

## Response to Letter to the Editors

# Response to comment on “Prevalence and factors associated with cryptococcal antigenemia among severely immunosuppressed HIV-infected adults in Uganda (Oyella et al. 2012)”

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We would like to thank you for the comments regarding our recent article: Cryptococcal antigenemia among severely immunosuppressed HIV-infected adults.

In response to your comment.

Sir, the recent report on “Prevalence and factors associated with cryptococcal antigenemia among severely immunosuppressed HIV-infected adults in Uganda” is very interesting [1]. Oyella *et al.* concluded that “Independent predictors of positive serum cryptococcal antigenemia were CD4+ T cell counts of less than 50 cells/mm, low body mass index, neck pain, signs of meningeal irritation, and a recent diagnosis of HIV infection” [1]. This work is a cross sectional study, not a case-control study; hence, there might be some bias on assessment of risk factor.

Our study design was cross-sectional study because it allows for determination of association between variables with no recall bias unlike case-control study, which is commonly used for rare diseases in a population and highly associated with recall bias. Cryptococcal infection in HIV-infected adults is very common in our setting. We only enrolled patients with no known history of cryptococcal infection.

In response to your comment regarding many other factors that might contribute to the cryptococcal antigenemia, we did put many variables to statistical test including gender and residence, which was statistically insignificant. But, there is still room for further work that may be done.

In response to your comment: “some identified factors (such as low CD4+ count and low body mass index) in this study being the same as the other reports whereas many factors are totally different” [2]: it is true that some of the factors are the same as findings in other studies. Our study has consolidated previous work done in this field and, on the other hand, identified factors seen in our setting because many of our patients present very late with advanced disease.

In response to your comment that there is no doubt that concurrent conditions might contribute to severe infection and this has not been completely investigated, we excluded majority of the patients with proven comorbidities in our study since most were on antiretroviral therapy (ART) and

were presenting with suspected immune reconstitution inflammatory syndrome. We potentially could have had higher cryptococcal antigenemia if we had included these patients. Clearly, further research needs to be done in individuals with concurrent conditions. As an example, there is an ongoing study through the Infectious Disease Institute in a rural hospital that is screening ART-naïve patients for cryptococcal antigenemia and a number of CRAG-positive subjects have died, not from cryptococcal infection but from TB co-infection.

In response to your comment about the quality control of the diagnostic test in this work:

The positive and negative controls included in the CRAG kits were tested in accordance with the manufacturers’ quality control protocol to ensure that the latex was functioning well during testing as outlined below.

All reagents and prepared samples were allowed to reach room temperature before use, and all procedures were performed at room temperature (21–25°C).

We used aseptic technique to avoid contamination of stock reagents with other or with test specimens, which could lead to erroneous results.

Tests were performed under careful standardized conditions with maintenance of latex suspension, volumes of reagents used and speed of rotation, reaction time and the degree of agglutination designated as a positive test.

Use of accepted microbiological practices for proper disinfection of potentially infectious material and contaminated equipment prior to disposal.

Glass slides were held at a slight angle above light and over a dark background for optimum ease of interpretation.

Also, some of the tests were repeated at Mulago National Referral and Teaching Hospital Core/Central laboratory for quality assurance purposes.

In response to your comment about the need to discuss the problem of false positive of the test kit [3] and of interest the false results occurring if improper transportation is applied [4]: we believe that false positive result is one of the limitations of the procedure; however, as with any diagnostic procedure, results obtained were evaluated in

light of clinical information and quality control standards were maintained. However, rheumatoid factor and other specimen components may interfere with the test. Specimens with obvious contamination and gross hemolysis were not used. Although control latex was used to identify the potential interferences, other procedural modification to eliminate the above included: pronase treatment and pre-treatment of specimen with heat.

The specimens were not transported using the BBL Port-A-Cul, which is highly associated with false positive results.

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